

Paradoxical High-Level Spiramycin Resistance and Erythromycin Susceptibility Due to 23S rRNA Mutation in Streptococcus constellatus

Agathe Capitaine, Paul-Louis Woerther, Michel Auzou, Elisabeth Chachaty, François Guerin, Jean-Christophe Giard, Vincent Cattoir, Christophe Isnard

▶ To cite this version:

Agathe Capitaine, Paul-Louis Woerther, Michel Auzou, Elisabeth Chachaty, François Guerin, et al.. Paradoxical High-Level Spiramycin Resistance and Erythromycin Susceptibility Due to 23S rRNA Mutation in Streptococcus constellatus. Microbial Drug Resistance, 2020, 26 (7), pp.727-731. 10.1089/mdr.2019.0288 . hal-02569879

HAL Id: hal-02569879 https://normandie-univ.hal.science/hal-02569879

Submitted on 9 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés. Paradoxical high-level spiramycin resistance and erythromycin
 susceptibility due to 23S rRNA mutation in *Streptococcus constellatus*

4

Agathe Capitaine¹, Paul-Louis Woerther², Michel Auzou¹, Elisabeth Chachaty³,
 François Guérin^{1,4}, Jean-Christophe Giard⁵, Vincent Cattoir^{6,7} and Christophe
 Isnard^{1,4,*}

8

9 Running title: Paradoxical MLS resistance due to 23S rRNA mutation in
 10 Streptococcus constellatus

11

Normandie Univ, UNICAEN, CHU de CAEN Normandie, Service de Microbiologie, 14000 12 Caen, France¹; Unité de Bactériologie-Hygiène, Département de Microbiologie, Assistance 13 Publique - Hôpitaux de Paris, Hôpital Henri Mondor, Créteil, France²; Service de 14 Microbiologie, Institut Gustave Roussy APHP, Villejuif, France³; Normandie Univ, 15 UNICAEN/UNIROUEN, EA2656 GRAM2.0, 14000 Caen, France 4; Normandie Univ, 16 UNICAEN, EA4655 U2RM, 14000 Caen, France⁵; Centre Hospitalier Universitaire de 17 Rennes, Site Pontchaillou, 35000 Rennes, France⁶; CNR de la Résistance aux Antibiotiques, 18 Laboratoire Associé « Entérocoques et résistances particulières chez les bactéries à Gram 19 positif », 35000 Rennes, France⁷. 20

21

22 *Correspondance : Mailing address: Dr. Christophe ISNARD, Service de
23 Microbiologie, CHU de CAEN, 14033 Caen Cedex, France.

24 E-mail: isnard-c@chu-caen.fr

25

26 **Keywords**: *Streptococcus constellatus*; *Streptococcus milleri* group; MLS resistance;

27 23S rRNA mutation; A2062C; ARMS qPCR.

Word counts: Abstract: 253; Text: 1942; Tables: 2; Figures: 1; References: 19

30 Abstract

31 **Objectives:** The aim of the study was to characterize phenotypically and 32 genotypically an uncommon mechanism of resistance to macrolides, lincosamides 33 and streptogramins (MLS) in a *Streptococcus milleri* group clinical isolate.

Methods: The isolate UCN96 was recovered from an osteoradionecrosis wound, and 34 35 was identified using the MALDI-TOF mass spectrometry and the partial sequencing of the sodA gene. Antimicrobial susceptibility testing (AST) were carried out by the 36 disc diffusion method and MICs were determined by the broth microdilution 37 technique. PCR screening was performed for MLS resistance genes described in 38 Gram-positive bacteria. Specific mutations in the ribosomal proteins L3-, L4- and 39 L22-encoding genes were also screened as well as those in domain V of the 23S 40 rRNA gene (rrl). The number of mutated copies of the rrl gene was determined using 41 ARMS (amplification-refractory mutation system) qPCR analysis. 42

Results: The clinical isolate UCN96 was unambiguously identified as *Streptococcus constellatus*. It was susceptible to all ML antibiotics except spiramycin (MIC >256 mg/L) while it was also resistant to streptogramins. Screening for all acquired resistance genes was negative while no mutation was found in genes coding for L3, L4 and L22 ribosomal proteins. Interestingly, a single mutation, A2062C (according to *Escherichia coli* numbering), was detected in the domain V of 23S rRNA.

Conclusion: Mutations at the position 2062 of 23S rRNA have been detected once
 in *S. pneumoniae*, and not yet in other *Streptococcus* spp. This mechanism is very

- 51 likely uncommon in Gram-positive bacteria since different copies of 23S rRNA
- 52 operons should be mutated for development of such a resistance pattern.

53 Introduction

Streptococcus constellatus belongs to the Streptococcus milleri group (SMG) besides 54 two others species, Streptococcus anginosus and Streptococcus intermedius. SMG 55 is part of the human microbiota and can be in the oral cavity as well as abdominal, 56 urogenital and upper respiratory tracts¹. Streptococci from the SMG are responsible 57 for various pyogenic infections including cardiac, skin, abdominal and central nervous 58 system infections¹. These organisms are usually susceptible to many antimicrobial 59 agents including those commonly used for infections caused by streptococci such as 60 β-lactams, macrolides-lincosamides-streptogramins (MLS) and glycopeptides. 61

62 Whereas there is no resistance to β -lactams and glycopeptides described in SMG, acquired MLS resistance has been reported, with approximately 17% of clinical 63 isolates resistant to erythromycin and clindamycin². In streptococci, MLS resistance 64 is mediated by two major mechanisms which are target site modification and active 65 drug efflux ³. Ribosomal alteration is caused by methylation of a specific adenine 66 residue (the so-called A2058) of target site in the 23S rRNA and is mediated by an 67 adenine-N6-methyltransferase encoded by a gene belonging to the erm class 68 (erythromycin ribosome methylase). This methylation of the A2058 residue induces a 69 conformational change in the 50S ribosomal subunit that alter the binding of 70 antibiotics to the ribosome and causes the so-called MLS_B phenotype. The second 71 mechanism consisted of an active efflux of the antibiotics encoded by the mef gene. 72 This active efflux is responsible for unique resistance to 14- and 15-membered 73 macrolides and confers the so-called M phenotype. Other mechanisms of MLS 74 resistance have been rarely described in Gram-positive bacteria, such as other 75 mutations in 23S rRNA or in L3, L4, L22 ribosomal proteins and to our knowledge, 76 have not been yet described in the SMG ^{4–8}. 77

The aim of this study was to decipher the molecular aspect of an atypical resistance phenotype in a clinical isolate of *S. constellatus* (UCN96).

80

(Part of this work was presented in the 14th European Congress of Clinical
 Microbiology and Infectious Diseases, EV0187, Copenhagen, 2015)

84 Materials & Methods

85 Bacterial strains used in the study

The UCN96 isolate was recovered in June 2014 from a osteoradionecrosis 86 radionecrosis wound in a patient hospitalized at the Gustave Roussy Institute 87 (Villejuif, France) for a tongue cancer medical history. The same bacterial strain was 88 recovered from the patient in a chin outpouring in May 2014 but the antimicrobial 89 susceptibility testing realized then, showed that the S. constellatus isolated was fully 90 susceptible to all MLS antibiotics. In front of this statement, the patient has received 91 pristinamycin at 1g twice in a day for 10 days since he was intolerant to β-lactams. 92 93 The isolate UCN96 was identified by MALDI-TOF mass spectrometry (Microflex; Brucker Daltonics; Germany) and partial sequencing of the sodA gene as previously 94 described ⁹. Staphylococcus aureus ATCC 29213 and Streptococcus pneumoniae 95 96 ATCC 49619 were used as antimicrobial susceptibility testing (AST) controls while an MLS-susceptible S. constellatus from our collection (S. constellatus 13422) was also 97 tested as wild-type control for comparative purposes. 98

99 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out by the disc diffusion method using 100 Mueller-Hinton agar plates supplemented with lysed horse blood (5%) and β-NAD 101 (20 mg/L) (bioMérieux; France) according to the Antibiogram Committee of the 102 French Society for Microbiology recommendations (CA-SFM/EUCAST; www.sfm-103 microbiologie.org). MICs of erythromycin, azithromycin, spiramycin, josamycin, 104 telithromycin. lincomycin, clindamycin, pristinamycin, quinupristin-dalfopristin, 105 quinupristin, dalfopristin and chloramphenicol were determined by the broth 106 microdilution technique according to EUCAST guidelines (http://eucast.org/). 107

109 DNA preparation, PCR assays and gene sequencing.

DNA extraction was performed using the automated easyMAG® extractor 110 (bioMérieux, France) according to the protocol suggested by the manufacturer. 111 Extracted bacterial DNA was eluted with 25 µl elution buffer and stored at -20°C. 112 PCR experiments for the detection of acquired genes putatively involved in MLS 113 resistance [e.g. erm(A), erm(B), erm(C), erm(F), erm(TR), erm(T), erm(X), msr(A) 114 and mef(A)] as well as uncommon acquired genes [Inu(A), Inu(B), Inu(C), Inu(E), 115 Isa(A), Isa(B), Isa(C), Isa(E), vat(A), vat(B), vat(C), vat(D), vat(E), vga(A), vga(B), 116 vga(C), vga(D), vga(E), vgb(A), vgb(B)] were carried out using an in-house method. 117 The screening of point mutations was also performed by PCR-sequencing using 118 specific primers (Sigma-Aldrich, France) (Table 1) allowing amplification of the L3, L4 119 and L22 ribosomal protein encoding genes (*rplC*, *rplD* and *rplV* genes respectively) 120 121 and in the 23S rRNA (rrl) gene. Amplification refractory mutation system (ARMS) gPCR was used to distinguished if the mutation of *rrl* gene affected all the alleles 122 harbored by the bacterial strain (e.g. 4 alleles in S. constellatus and all SMG) as 123 previously described in eucaryotes ^{10,11}. Primers used for qPCR ARMS experiment 124 are detailed in Table 1. Briefly, two sets of primers were design. Oligonucleotide 125 sequences of the forward primers were strictly complementary to the *rrl* region where 126 the mutation was found. For one of the two forward primers, the nucleotide localized 127 at the 3' end corresponded to the nucleotide found in the mutant strain sequence, 128 whereas for the other, it corresponded to the nucleotide found in the wild type. The 129 reverse primer was the same for the 2 experiments. ARMS qPCR experiments were 130 made by real-time PCR using the SsoAdvanced universal SYBR green supermix kit 131 and a CFX Connect real-time PCR detection system (Bio-Rad, France) according to 132 manufacturer's recommendations. To determine if the mutation appeared in several 133

gene loci, a comparison of cycle threshold between the two sets of primers was analyzed for the mutated strain UCN96. A susceptible strain of our collection, *S. constellatus* 13422, was used as comparator. In case of higher initial amount of mutated DNA matrix, a lower CT value should be obtained. Then if a difference in CT values was observed between the 2 sets of primers, we should conclude that more than one allele was mutated. Conversely, the same result was expected in favor of the "wild-type" set of primers when using the *S. constellatus* 13244 DNA as template.

141

142 Multiple alignment, phylogenetic and gene analysis.

143 Genome of the annotated strain S. constellatus 1050 available at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/genome/) was used for 144 comparative sequences analysis. 23S rRNA gene partial sequence was then 145 compared to deposited sequences available from the BIBI site database 146 (https://umr5558-bibiserv.univ-lyon1.fr/lebibi/lebibi.cgi). Sequence comparison by 147 multiple alignment and phylogenetic analysis were performed using BioEdit 148 Sequence Alignment Editor Software (http://www.mbio.ncsu.edu/bioedit/bioedit.html) 149 neighbour-joining and the algorithm with the ClustalOmega software 150 151 (http://www.ebi.ac.uk/Tools/msa/clustalo/).

153 **Results**

154 Bacterial Identification

The identification of strain UCN96 was carry out twice using the MALDI-TOF mass spectrometry technology and was unambiguously confirmed as *S. constellatus* by the partial *sodA* gene sequencing.

158

159 In vitro antimicrobial susceptibility testing

By the disc diffusion method, the strain UCN96 was susceptible to almost all tested 160 ampicillin, kanamycin, antimicrobials: penicillin G, tobramycin, gentamicin, 161 162 vancomycin, teicoplanin, levofloxacin, rifampicin, tetracycline, chloramphenicol and cotrimoxazole but in contrast, it exhibited an uncommon MLS resistance. After MIC 163 determination, the strain appeared harbouring an a priori paradoxical MS_{AB} 164 165 resistance phenotype. Indeed, the strain was susceptible to all 14- and 15-membered macrolides (e.g. erythromycin, telithromycin, and azithromycin, with MICs of 0.03, 166 <0.008 and 0.12 mg/L, respectively), lincosamides (e.g. lincomycin and clindamycin, 167 with MICs of 1 and 0.06 mg/L, respectively) and chloramphenicol with a MIC of 8 168 mg/L. On the other hand, it was highly-resistant to spiramycin or josamycin (16-169 membered macrolides) with MICs of >256 and 32 mg/L, respectively and 170 streptogramins (MIC >64 mg/L; 8 mg/L, 4 mg/L and 8 mg/L for dalfopristin, 171 quinupristin, quinupristin-dalfopristin and pristinamycin, respectively.) (Table 2). As 172 compared to S. constellatus 13422 susceptible strain, UCN96 exhibited MICs of 173 spiramycin, josamycin, dalfopristin, quinupristin, quinupristin-dalpristin 174 and pristinamycin 4, 512, 32, 8, 8 and 64-fold higher, respectively (Table 2). 175

176

177 Molecular characterization of the uncommon resistance phenotype

PCR experiments for the detection of acquired genes putatively involved in MLS 178 179 resistance as well as uncommon acquired resistance patterns were all negative and no mutation was found in L3, L4 and L22 ribosomal protein-encoding genes. 180 Interestingly, the strain displayed a point mutation in the *rrl* gene at an unexpected 181 position. Indeed, a nucleotide transition was found at position 2062 (E. coli 182 numbering) (A2062C) as shown in figure 1A. Considering that S. constellatus 183 harbored four copies of the ribosomal operon we assessed if the mutation affected 184 one or several *rrl* operons. The ARMS gPCR technique was used and confirmed the 185 presence of the A2062C mutation in more than a single copy of the 23S rRNA gene 186 187 in the strain UCN96. When DNA extracted from the resistant strain UCN96 was used as template, cycle threshold (CT) obtained with the set of mismatches primers 188 appeared sooner (e.g. fourteen times), than the CT obtained with the set of primers 189 190 designed with the wild type sequence of the *rrl* operon (Figure 1B right). Inversely, when the DNA extracted from the susceptible strain was used as template, only a six 191 192 CT difference was observed between the two sets of primers in favor of the unmismatches primers (Figure 1B left). Such results, allow us to strongly suggest 193 that the point mutation A2062C was present in more than one 23S rRNA operon 194 195 copy.

197 **Discussion**

S. constellatus can be found as a part of the oral cavity and upper respiratory tract flora but can be also responsible for various pyogenic infections where a therapeutic management is required ¹. β-lactams are the first-line molecules recommended for the treatment of streptococcal infections but in patients intolerant to that class of antibiotics, MLS antimicrobials constitute an alternative therapy.

The target site for MLS molecules is the 50S ribosomal subunit ¹² and many cases of 203 MLS resistance could be linked with an alteration by methylation of 23S rRNA at 204 positions 2058 and/or 2059 ^{3,6}. In bacteria harboring several rRNA operon copies 205 (such as Staphylococcus spp., Streptococcus spp. or Enterococcus spp.) the most 206 prevalent mechanism for MLS resistance is an acquired mechanism consisting in 207 expression of erm gene encoding methyltransferase or active efflux of the antibiotics 208 encoded by the *mef* gene ^{3,7,13,14}. Point mutations in 23S rRNA have been reported 209 and appeared at specific positions of the domain V especially in pathogens harboring 210 only 1 or 2 rRNA operon copies, such as mycoplasma with a prevalence in 211 Mycoplasma genitalium estimated around 50%¹⁵. These mutations, regarding the 212 species and nucleotide substitution, conferred different phenotypes of resistance. To 213 our best knowledge, MLS resistance linked to these types of mutations seemed to be 214 less prevalent in Gram-positive bacteria likely because they harbored a higher 215 number of rRNA operons (4 rrn operons in streptococci or enterococci for example) 216 and appeared generally at position 2058, 2059 or 2611, according to the species 217 involved ⁷. Interestingly, in our *S. constellatus* clinical isolate, the mutation found 218 (A2062C) seemed to be very uncommon since it has been reported only once in a 219 spiramycin- and streptogramin-resistant strain of Streptococcus pneumoniae and in a 220 chloramphenicol-resistant Halobacterium halobium ^{16,17,18}. Surprisingly, whereas H. 221

halobium harbored a single copy of rRNA operon, S. pneumoniae harbored 4 copies 222 (like S. constallatus) and the mutation was found in the four copies of this operon 223 ^{16,18,19}. Our data were in accordance with these observations since several copies of 224 rrl operon seemed altered in S. constellatus UCN96. Then, clinical isolates of 225 viridans streptococci that harbored this unusual phenotype of resistance 226 (susceptibility to 14-15 membered macrolides and lincosamides, resistance to 16-227 membered macrolides and streptogramins) has not been reported yet. Moreover, as 228 previously described by different studies concerning the crystal structure of the 229 interaction area of MLS antibiotics with 50S SU, amino acids modification in position 230 2062 (transition to a nonpolar to a polar amino acid) possibility changed hydrogen 231 links between MLS antibiotics and their target in 50S SU of *Staphylococcus aureus* 232 ribosome. As a consequence, resistance to different MLS antibiotics should be 233 explained by this conformational variation²⁰. 234

As a conclusion, we have identified and characterized a point mutation (A2062C) 235 236 found in more than one locus of the domain V of 23S rRNA that confers spiramycin and streptogramins resistance in S. constellatus isolated from a patient suffering of 237 radionecrosis wound and treated with pristinamycin. Since S. constellatus is part of 238 the oral cavity and upper respiratory tract flora and that irradiation is largely use in 239 head and neck cancers, these data suggest that in clinical strains of SMG in patients 240 that previously received MLS therapy, it would be wise to follow the susceptibility of 241 the strain concerning all the MLS class of drugs (e.g. 14-, 15- and 16- membered 242 macrolide, ketolide and also to lincosamide and streptogramins). 243

245 Author Disclosure Statement

The authors declare that they have no conflicts of interest.

- 248 Funding
- 249 This work was supported by a grant from the Ministère de l'Enseignement Supérieur
- et de la Recherche to EA4655, Université de Caen Normandie, France.
- 251

252 **References**

Gossling, J. 1988. Occurrence and pathogenicity of the *Streptococcus milleri* group. Rev. Infect. Dis. 10:257–285.

Limia, A., M.L. Jiménez, T. Alarcón, and M. López-Brea. 1999. Five-year
 analysis of antimicrobial susceptibility of the *Streptococcus milleri* group. Eur. J. Clin.
 Microbiol. Infect. Dis. Off. Publ. Eur. Soc. Clin. Microbiol. 18:440–444.

Leclercq, R. 2002. Mechanisms of resistance to macrolides and
 lincosamides: nature of the resistance elements and their clinical implications. Clin.
 Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 34:482–492.

Cattoir, V., L. Merabet, P. Legrand, C.-J. Soussy, and R. Leclercq. 2007.
 Emergence of a *Streptococcus pneumoniae* isolate resistant to streptogramins by
 mutation in ribosomal protein L22 during pristinamycin therapy of pneumococcal
 pneumonia. J. Antimicrob. Chemother. 59:1010–1012.

Bingen, E., R. Leclercq, F. Fitoussi, N. Brahimi, B. Malbruny, D. Deforche,
 and R. Cohen. 2002. Emergence of group A Streptococcus strains with different
 mechanisms of macrolide resistance. Antimicrob. Agents Chemother. 46:1199–1203.

Weisblum, B. 1995. Erythromycin resistance by ribosome modification.
 Antimicrob. Agents Chemother. 39:577–585.

270 7. Vester, B., and S. Douthwaite. 2001. Macrolide resistance conferred by base
271 substitutions in 23S rRNA. Antimicrob. Agents Chemother. 45:1–12.

Malbruny, B., A. Canu, B. Bozdogan, B. Fantin, V. Zarrouk, S. DutkaMalen, C. Feger, and R. Leclercq. 2002. Resistance to quinupristin-dalfopristin due
to mutation of L22 ribosomal protein in *Staphylococcus aureus*. Antimicrob. Agents

275 Chemother. 46:2200–2207.

Poyart, C., G. Quesne, S. Coulon, P. Berche, and P. Trieu-Cuot. 1998.
 Identification of Streptococci to species level by sequencing the gene encoding the
 manganese-dependent superoxide dismutase. J. Clin. Microbiol. 36:41–47.

Newton, C.R., A. Graham, L.E. Heptinstall, S.J. Powell, C. Summers, N.
Kalsheker, J.C. Smith, and A.F. Markham. 1989. Analysis of any point mutation in
DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Res.
17:2503–2516.

11. Ye, S., S. Dhillon, X. Ke, A.R. Collins, and I.N. Day. 2001. An efficient
procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Res.
29:E88-88.

Wilson, D.N. 2014. Ribosome-targeting antibiotics and mechanisms of
bacterial resistance. Nat. Rev. Microbiol. 12:35–48.

13. Roberts, M.C. 2008. Update on macrolide-lincosamide-streptogramin,
ketolide, and oxazolidinone resistance genes. FEMS Microbiol. Lett. 282:147–159.

Lucier, T.S., K. Heitzman, S.K. Liu, and P.C. Hu. 1995. Transition mutations
in the 23S rRNA of erythromycin-resistant isolates of *Mycoplasma pneumoniae*.
Antimicrob. Agents Chemother. 39:2770–2773.

Deguchi, T., M. Yasuda, K. Horie, K. Seike, M. Kikuchi, K. Mizutani, T.
Tsuchiya, S. Yokoi, M. Nakano, and S. Hoshina. 2015. Drug resistance-associated
mutations in *Mycoplasma genitalium* in female sex workers, Japan. Emerg. Infect.
Dis. 21:1062–1064.

16. Tait-Kamradt, A., T. Davies, P.C. Appelbaum, F. Depardieu, P. Courvalin,

J. Petitpas, L. Wondrack, A. Walker, M.R. Jacobs, and J. Sutcliffe. 2000. Two
 new mechanisms of macrolide resistance in clinical strains of *Streptococcus pneumoniae* from Eastern Europe and North America. Antimicrob. Agents
 Chemother. 44:3395–3401.

302 17. Depardieu, F., and P. Courvalin. 2001. Mutation in 23S rRNA responsible for
 303 resistance to 16-Membered macrolides and streptogramins in *Streptococcus* 304 *pneumoniae*. Antimicrob. Agents Chemother. 45:319–323.

Mankin, A.S., and R.A. Garrett. 1991. Chloramphenicol resistance mutations
in the single 23S rRNA gene of the archaeon *Halobacterium halobium*. J. Bacteriol.
173:3559–3563.

19. Klappenbach, J.A., P.R. Saxman, J.R. Cole, and T.M. Schmidt. 2001. *rrn*db: the ribosomal RNA operon copy number database. Nucleic Acids Res.
29:181–184.

Matzov, D., Z. Eyal, R.I. Benhamou, M. Shalev-Benami, Y. Halfon, M.
Krupkin, E. Zimmerman, H. Rozenberg, A. Bashan, M. Fridman, and A. Yonath.
2017. Structural insights of lincosamides targeting the ribosome of *Staphylococcus aureus*. Nucleic Acids Res. 45:10284–10292.

315

316

317 Legend of the figure

318

Figure 1. A) Partial nucleotide sequence alignment of 23S rRNA (*rrl*) encoding gene.
Mutation at position 2062 is framed in bold line (*E. coli* numbering). B Left) RT-PCR
results curves showing CT difference between wild-type (WT) primers vs ARMS
Capitaine et al.

mismatches primers for a MLS susceptible strain of *S. constellatus* 13422 and the resistant strain UCN96. Green lines corresponding to amplification when using WT primers and red line corresponding to amplification when using mismatches primers.

```
23S_S. constellatus UCN96
23S_S. constellatus 1050
23S_S. pneumoniae
23S_E. coli
```

CGGAAAGCCCCATGGAGCTTTACTGCAGTTTGATATTGAGTGTCTGTACCACATGTACA CGGAAAGACCCCATGGAGCTTTACTGCAGTTTGATATTGAGTGTCTGTGCCACATGTACA CGGAAAGACCCCCATGGAGCTTTACTGCAGTTTGATATTGAGTGTCTGTACCACATGTACA CGGAAAGACCCCGTGAACCTTTACTATAGCTTGACACTGAACATTGAGCCTTGATGTGTA

2062

A)





S. constellatus UCN96



Gene		Purpose				
(ribosomal protein)	Designation	Sequence (5' to 3')				
rp(C (1 2)	L3-F	CGCCCTTGGTATCTTAATCT	Detection of point mutation in			
rpic (L3)	L3-R	CTTCACCAGCTTCTTTACCAG	L3 protein			
rp(D (1 4)	L4-F	GTGCCTGGTGCTAAGAAATC	Detection of point mutation in			
<i>τριο</i> (L4)	L4-R	TCTTCAAGAAGAGCCATTGA	L4 protein			
rn/\//1.22)	L22-F	CACAAGCTTGGTGAGTTTGC	Detection of point mutation in			
rprv (L22)	L22-R	TACCATTTGGCATCCCAGTC	L22 protein			
<i>rrl</i> (23S rRNA)	23S-F	CGAAATTCCTTGTCGGGTAA	Detection of point mutation in			
domain V	23S-R	CCGTAGATGATCAACCTAC	domain V of 23S rRNA			
	23S_ARMS_WT_F1	CGCGACAGGACGGAAAGA				
rrl (23S rRNA)	23S_ARMS_WT_F2	CGCGACAGGACGGAATGA	Allelic determination of point			
	23S_ARMS_Mut_F1	CGCGACAGGACGGAAAGC	mutation in 23S rRNA			
domain V	23S_ARMS_Mut_F2	CGCGACAGGACGGAATGC	(ARMS)			
	23S_ARMS_R	ATCCCAACATCGCCTCCATC				

Table 1. Primers used in this study

	MIC (µg/ml)											
Strains	Macrolides			Lincosamides				Streptogramins			Phenicols	
	ERY	TEL	AZI	SPI	JOS	LIN	CLI	DAL	QUI	QD	PRI	CHL
S. constellatus 13422	<0.01	<0.01	0.06	0.06	0.06	0.5	0.03	2	1	0.5	0.12	2
S. constellatus UCN96	0.03	<0.01	0.12	≥256	32	1	0.06	≥64	8	4	8	8
S. aureus 29213	0.25	0.06	1	8	2	1	0.25	4	2	0.5	0.25	8
S. pneumoniae 49619	0.06	<0.01	0.12	0.25	0.06	0.5	0.06	16	1	0.25	0.12	2

Table 2. MICs of MLS antibiotics and Chloramphenicol used in this study

ERY, Erythromycin; TEL, Telithromycin; AZI, Azithromycin; SPI, Spiramycin; JOS, Josamycin; LIN, Lincomycin; CLI, Clindamycin;

DAL, Dalfopristin; QUI, Quinupristin; Q-D, Quinupristin-Dalfopristin; PRI, Pristinamycin; CHL, Chloramphenicol.